Comparison of volatile leaf terpenoids from *Juniperus monosperma* and *J. osteosperma* leaves: intact, ground and exposed to ambient temperature.

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ABSTRACT

The effects of sample preparation on yields and composition of volatile terpenoids were examined for oils from *Juniperus monosperma* and *J. osteosperma* leaves obtained by 24 h steam distillation from intact, ground-frozen, ground- 4h RT, and ground-18h RT leaves. For *J. monosperma*, the total oil yield was largest from ground-frozen (4.48%), then declined in the 4h RT (4.19%) and 18 h RT (2.51%) treatments, with yield from intact leaves being intermediate (3.46%). The major component, α-pinene, declined from 22.7 mg/g to 5.8 mg/g upon exposure to RT for 18h. For *J. osteosperma*, the total oil yield was also largest from the ground-frozen (8.9%), then declined in the 4h RT (5.3%) and 18 h RT (4.7%) treatments, with yield from intact leaves being intermediate (5.63%). The major component, bornyl acetate declined from 14.4 mg/g to 10.0 mg/g upon exposure to RT for 18h. Sabinene declined from 11.3 mg/ g to 3.5 mg/g after exposure to RT for 18h. The leaf oils of *J. osteosperma*, having much less volatile monoterpenes and with oil glands deeply embedded in its leaves, were much less affected by exposure to RT for 18h. Published on-line **www.phytologia.org** *Phytologia 96(3): 207-217 (July 1, 2014).* ISSN 030319430

KEY WORDS: *Juniperus monosperma, J. osteosperma, Neotoma stephensi,* woodrats, terpene distillation, ground leaves, exposure to ambient.

There have been several studies on the effects of leaf storage at ambient (room temperature, RT) on the volatile leaf oil yields and composition of *Juniperus* (*J. thurifera*, Achak, et al., 2008; *J. excelsa*, Shanjani et al., 2010; *J. pinchotii, J. virginiana*, Adams, 2010; 2011; 2012a; 2013a, b). Adams (2012a) reported the oil yields of *J. virginiana* varied non-significantly between fresh leaves and those stored for up to 18 mo. at RT, but oil yields significantly declined between 18 and 25 mo. at RT. The major monoterpenes, sabinene and limonene, were stable for up to 8 mo., then significantly declined at 18 mo. and 25 mo. (Adams, 2012a). It might be noted that the volatile leaf oil is stored in oil glands. and the oil glands in the leaves of *J. virginiana* are embedded (sunken) in the leaves and do not rupture. In a study of *J. pinchotii*, a species with ruptured oil glands, Adams (2013b) found little significant variation in oil yields between fresh leaves and those stored up to 24 mo. at RT. The major monoterpene, sabinene, was stable for 4 mo. at RT (113 - 103 mg/g), then declined between 4 and 8 mo, then remained stable (82.2, 73.5, 80.4 mg/g) in the 8, 16 and 24 mo. at RT samples. The major oil component, camphor (ranging from 40 - 31%), declined initially from 300 mg/g (fresh leaves) to 209 mg/g (0.5 mo. at RT), then remained steady (no significant differences) from 0.5 to 24 mo. at RT (Adams, 2013b). However, all the afore-mentioned studies examined the effects of storage on volatile leaf oils of *Juniperus* using intact

leaves. None of these studies examined the effects of leaf grinding on volatile leaf oil stability during storage.

Juniperus is considered a poor forage for most mammals due to the presence of terpenes that can act as feeding deterrents (Gershenzon and Dudareva, 2007). Terpenes also have numerous toxic effects on mammals such as central nervous system depression, contact dermatitis, lung function impairment, liver and kidney cysts and even death (Sperling et al., 1967; Savolainen, 1978; Falk et al., 1990). Despite this, there are multiple species of woodrats (genus *Neotoma*) that consume juniper. *Neotoma stephensi* specializes on *Juniperus monosperma; N. albigula* consumes *J. monosperma* and *J. osteosperma;* and *N. lepida* consumes *J. osteosperma* (Vaughan, 1982; Torregrossa and Dearing, 2009; Magnanou et al., 2009). For example, browsing patterns on *J. monosperma* by the specialist *N. stephensi*, do not seem to be driven by terpene content (Adams et al., 2014) probably due to the animals' efficient physiological mechanisms to deal with the terpenes present (Boyle and Dearing 2003; Sorenson et al., 2004; Skopec et al., 2007; Skopec and Dearing, 2011; Torregrossa et al., 2011). Understanding the physiological and behavioral adaptations that allow these woodrat species to consume juniper may provide insight on ways to improve other mammalian species' performance on juniper. Juniper encroachment into rangelands is a major concern in the American West and increasing the voluntary intake of juniper by sheep or goats is proposed as a viable biocontrol tool (Estell et al. 2014a,b, Utsumi et al., 2013).

When feeding juniper leaves mixed with other feedstocks, under lab conditions, it is important that juniper and other feedstocks be finely ground and mixed, so woodrats (or other animals under consideration) do not intentionally select for certain feed components. However, merely grinding juniper leaves appears to release terpene volatiles as some oil glands are ruptured during grinding. In addition, as the feed is served at room temperature (RT), additional volatiles are likely lost during the course of a feeding trial. The purpose of the present paper is to report on the effects of sample preparation on yields and composition of volatile terpenoids from *Juniperus monosperma* and *J. osteosperma* leaves obtained by 24h steam distillation of leaves from four treatments: intact, ground-frozen, ground- 4h RT, and ground-18h RT.

MATERIALS AND METHODS

Plant material: Juniperus monosperma - a bulk collection was made by K. Kohl (2014) 35° 26.708' N; 111° 21.572' W, elev. 5290 ft, November, 2013, Coconino Co., AZ.
Leaf material subsamples (approx. 50 g FW) treated as: (Adams lab accession)
Adams 14209, intact fresh leaves.
Adams 14210, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, then frozen immediately.
Adams 14211, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, exposed to RT, 4h, then frozen.
Adams 14212, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, exposed to RT, 18h, then frozen.
J. osteosperma - a bulk collection was made by K. Kohl (2014) 40° 19'N 112° 54'W, 5650 ft, White Rocks, Tooele Co., UT.

Leaf material subsamples (approx. 50 g FW) treated as: (Adams lab accession)
Adams 14213, intact fresh leaves.
Adams 14214, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, then frozen immediately.
Adams 14215, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, exposed to RT, 4h, then frozen.
Adams 14216, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, exposed to RT, 18h, then frozen.

Essential oils analysis - A portion (50 g FW) of the fresh foliage was kept cold (-20°C) and in the dark; then the leaves with 2 mg of methyl decanoate added (as an internal standard) were exhaustively steam-distilled for 24 h using a modified circulatory Clevenger-type apparatus (Adams 1991). Oil samples were concentrated (diethyl ether trap-removed) with nitrogen and stored at -20°C until analyzed. Steam distilled leaves were oven dried to a constant weight (48 hr, 100°C) for the determination of oil yield as [oil wt./(oil wt. + oven dried extracted foliage wt.)]. The extracted oils were analyzed on a HP5971 MSD mass spectrometer: 0.2 ul of a 10% solution (in diethyl ether) oil injected, split, 1:10, temperature programmed, linear, 60° - 246°C at 3°C/min. (62 mins.), carrier gas He, flow 34.96 cm/sec or 1.02 ml/min, injector 220°C, detector 240°C, scan time 1/sec, directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25-micron coating thickness, fused silica capillary column (see Adams 2007, p. 4, for detailed operating conditions). Identifications were made by searches of our volatile oil library (Adams 2007) using HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantification was by flame ionization detector on an HP 5890 gas chromatograph operated under the same conditions as the GCMS (above) using the HP Chemstation software.

Statistical analyses - Terpenoids (as percentage of total oil and as mg per g dry foliage weight) were compared among the samples by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Differences were considered significant at $P \leq 0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

Comparisons between the yields and compositions of intact leaves, ground-frozen, ground-4h RT, and ground-18 RT materials are given in Table 1. Notice the oils are much easier to distill from ground-frozen than intact leaves (3.26% intact, 4.48%, ground-frozen). In *Juniperus*, the leaf oil is sequestered in oil glands (Fig. 1). The oil glands of *J. monosperma* are near the leaf surface as in *J. californica* and *J. occidentalis* (Fig. 1). It is not surprising that ground leaves release the oils more readily than intact leaves.

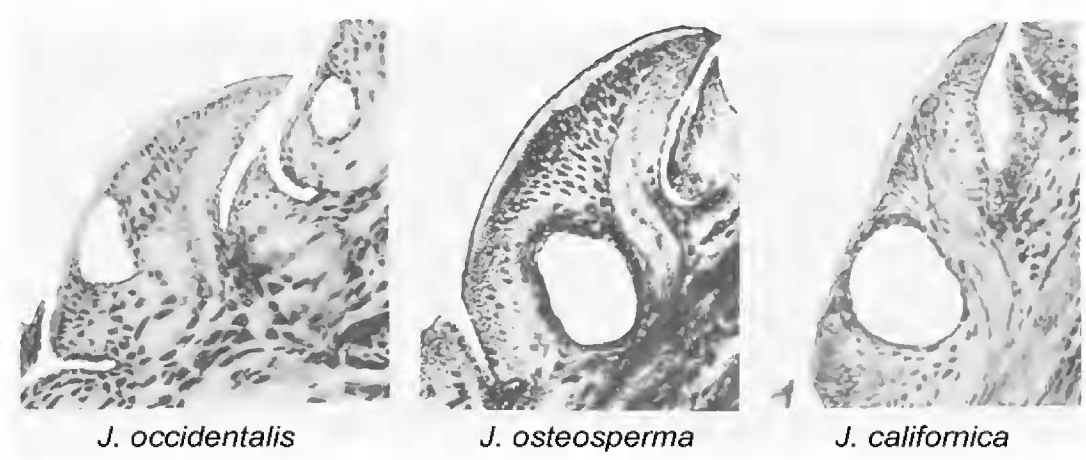

Figure 1. Leaf cross-sections for *J. occidentalis, J. osteosperma* and *J. californica* (from Frank Vasek, pers. communication). Notice for *J. occidentalis*, which has conspicuous and ruptured oil glands, the gland is at the leaf surface. The oil glands of *J. californica* are conspicuous, and occasionally ruptured. The oil glands in *J. osteosperma* are not conspicuous nor ruptured and are embedded in the leaves. The oil glands of *J. monosperma* (not shown) are near the leaf surface as in *J. californica*, conspicuous and often ruptured.

Oil yields and α-pinene (% data) show (Fig. 2) similar patterns. Oil and α-pinene yields are both larger in ground leaves than intact leaves. Comparing ground-frozen, ground-4h RT and ground-18h RT shows a significantly different decline between ground-4h RT and ground-18h RT treatments. This same pattern was observed in the other monoterpenes. Examination of yields and α-pinene (mg/g basis) shows a similar pattern (Fig. 3) to the % data (Fig. 2), except the decease in α-pinene in the mg/g data is not nearly as severe as in the % data.

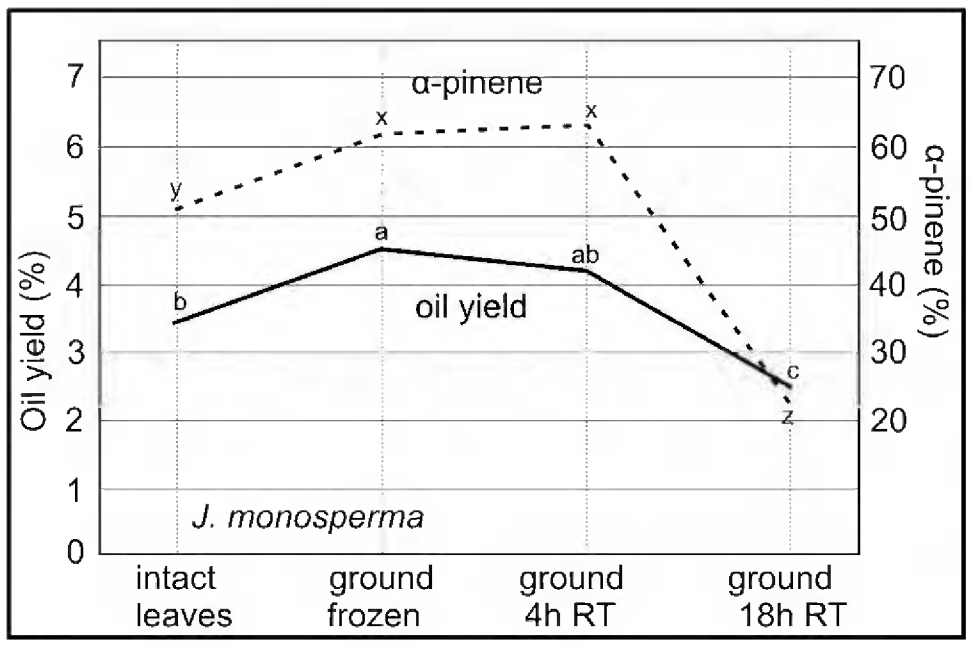

Fig. 2. Oil yields and α-pinene (% total oil, DW basis) of *J. monosperma*. Any data points on a line with a different letter are significantly different.

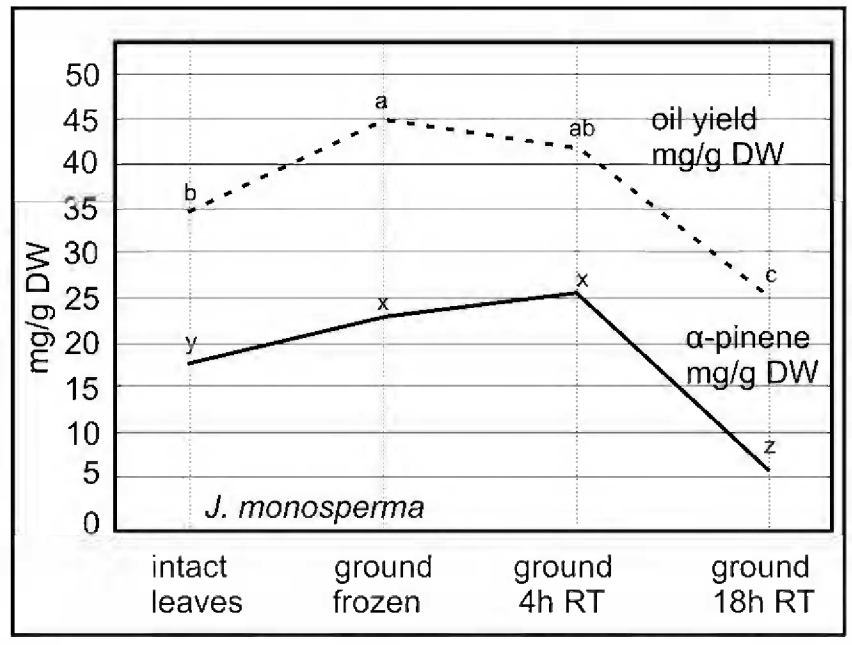

Fig. 3. oil and α-pinene of *J. monsperma*. (mg/g DW basis)

The β-eudesmol and elemol, sesquiterpenes, being less volatile than monoterpenes, displayed the opposite pattern (Fig. 4, % data). The decline in these sesquiterpenes from intact leaves and ground-frozen leaves appears to be due to the higher efficiency of removal of the more volatile monoterpenes from the ground-frozen leaves. The concentration of the sesquiterpenes was relatively larger in the ground-18h RT sample due to the loss of the more volatile monoterpenes during exposure of 18h at RT. A similar pattern is seen on a mg/g DW basis (Fig. 5), but less of a change between 4h RT, and 18h RT as β-eudesmol and elemol are not very volatile.

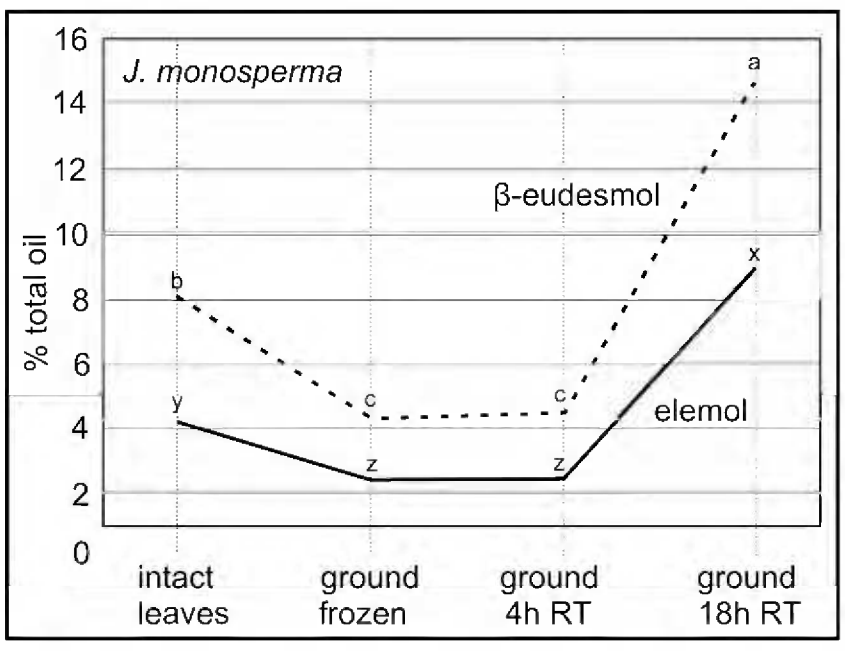

Fig. 4. Changes in β-eudesmol and elemol in the treatments. Note the large increase in the ground-18h RT sample. (% total oil basis)

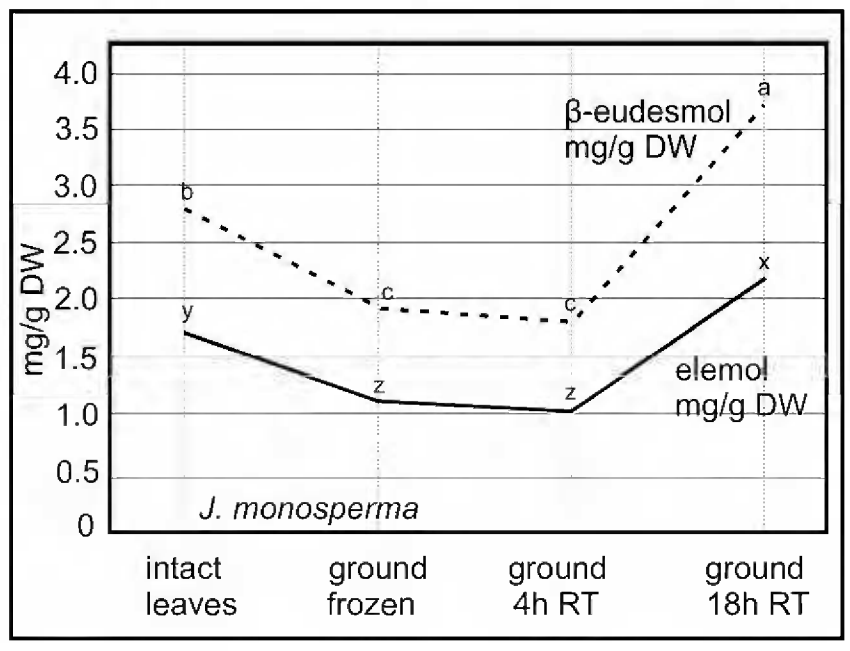

Fig. 5. Graph of β-eudesmol and elemol (mg/g DW basis). The increase from ground-4h RT to 18h RT is less than in the % total oil data (Fig. 4).

Although leaf browning (or yellowing) is visible after 4h RT (Fig. 6), the browning is more advanced in the 18h RT sample (Fig. 6). The browning is likely due to the oxidation of phenolics by polyphenol oxidase. However, no new components were found in the leaf oils (Table 1). It appears that the terpenoids extracted in this study are not susceptible to this type of oxidation and exposure to RT for up to 18h does not seem to induce any artifacts into the oil, except for the loss of the more volatile monoterpenes.

Fig. 6. Colors of ground-frozen, ground-4h RT and ground-18h RT leaves.

The composition of the oil from intact leaves of *J. osteosperma*, Utah, is shown in Table 2. It might be noted that the composition of this oil differed somewhat from the recent leaf oil report (Adams, 2012b, 2h distillation). This is likely due to difference in distillation time (24h vs. 2h), and geographic variation in *J. osteosperma* leaf oils. Yet, it is notable that the present *J. osteosperma* oil contained only a small amount of camphor (5.1%) compared to 16 to 60% camphor reported by Adams (2012b).

The trend in oil yields for *J. osteosperma* (Table 2) is similar to that found for *J. monosperma* (Table 1) with intact leaves yielding less oil than ground-frozen leaves (5.63 vs. 8.90%, Table 2). However, there is very large drop in oil yields from ground-frozen (8.90%) to ground-4h RT (5.30%) and ground-18h RT (4.7%), compared to a modest decline in *J. monosperma* from the ground-frozen (4.48%) to the ground-4h RT (4.19%). Because the oil glands of *J. osteosperma* are embedded in the leaves (Fig. 1), this could account for the higher efficiency of oil distillation from ground leaves (8.90%) than from intact leaves (5.63%).

Yields for α-pinene and sabinene show the same pattern for the four treatments: an increase in the ground-frozen leaves, then a decline with exposure to RT conditions (Fig. 7, % total oil basis). A similar pattern is seen on a mg/g DW basis (Fig. 8), except α-pinene, and sabinene show a greater decline from ground-frozen to ground-4h RT treatments than found in the % total oil data (Fig. 7).

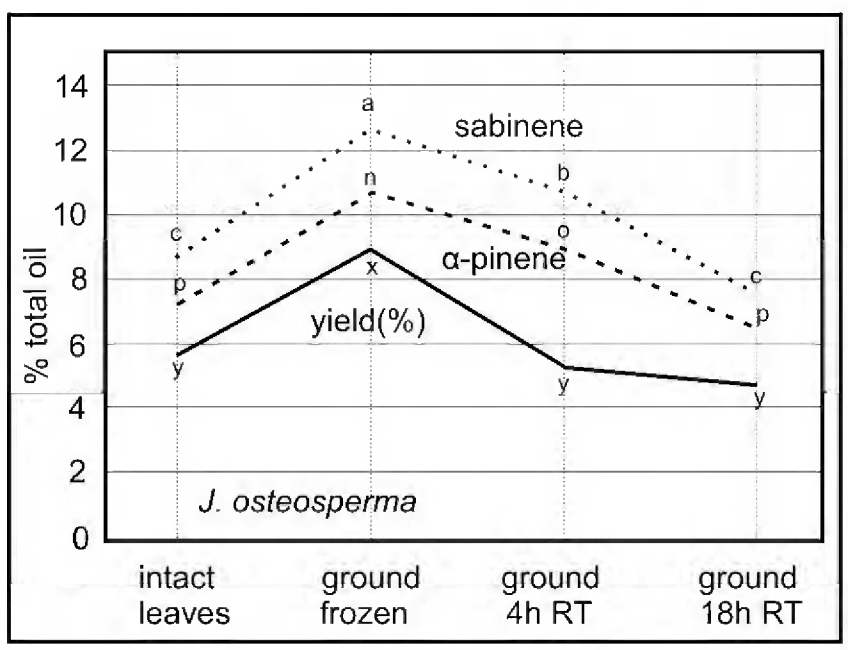

Fig. 7. Changes in oil yield, α-pinene, and sabinene (% total oil basis).

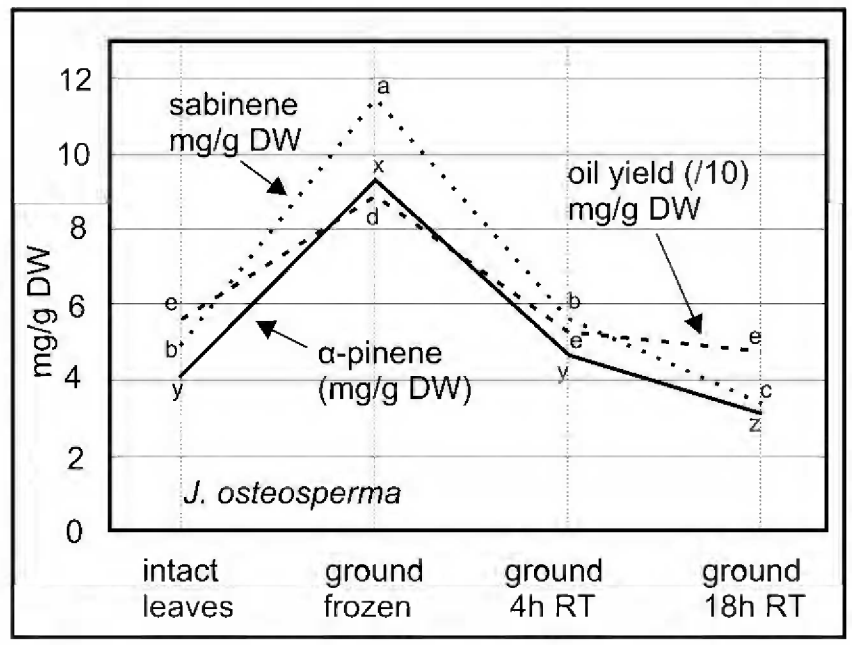

Fig. 8. Variation in oil yield, α-pinene, and sabinene (mg/g DW basis).

The major oxygenated terpenoids and sesquiterpenoids, bornyl acetate, camphor and elemol, display a different pattern (Fig. 9, % total oil basis). Intact leaves yielded more of these compounds,

decreasing in the ground-frozen leaf extract (Fig. 9). Bornyl acetate and camphor both increased as a proportion the total oil upon exposure to RT (Fig. 9). However, on a mg/g DW basis, larger amounts bornyl acetate and camphor were obtained from ground-frozen samples than from intact leaves. This, of course, reflects the overall greater yields obtained from ground leaves (Fig. 7). It is interesting that the yields of elemol, a less volatile sesquiterpene alcohol, was not much influenced by grinding or exposure to RT conditions (Figs. 9, 10).

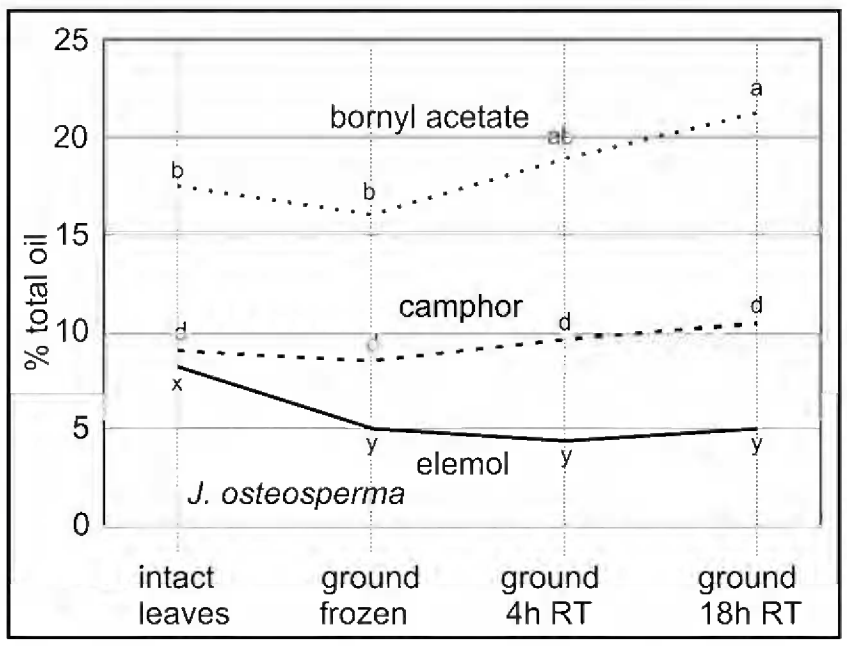

Fig. 9. Variation in bornyl acetate, camphor and elemol (% total oil basis) among treatments.

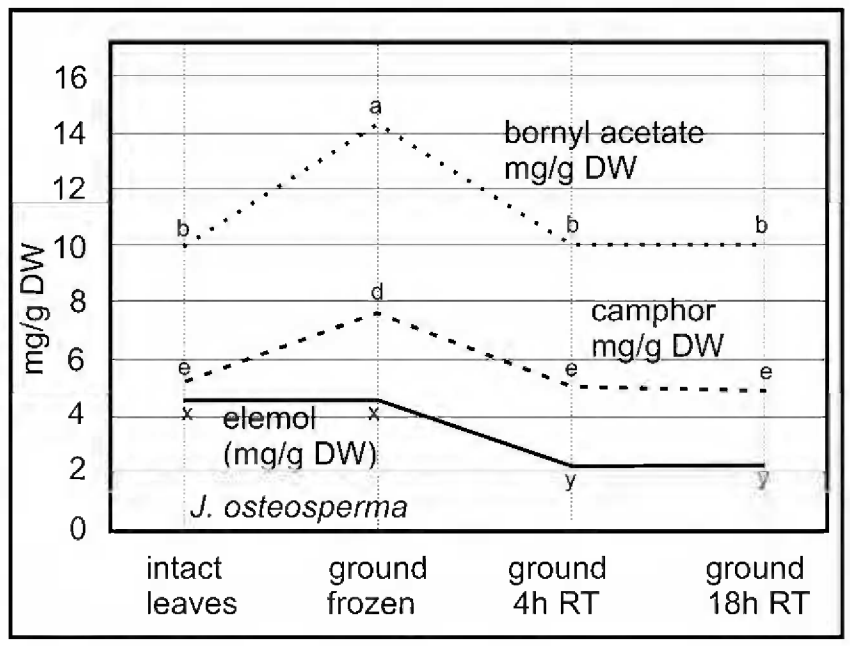

Fig. 10. Changes in bornyl acetate, camphor and elemol (mg/g DW basis).

The volatile leaf terpenoids of *J. osteosperma*, as previously seen in *J. monosperma*, are impacted by exposure to RT conditions for 4h and 18h. However, on a mg/g DW basis, the amount of terpenes lost from *J. osteosperma* ground leaves appears to be less than found in *J. monosperma*. This seems likely due to the embedded oil glands and the lesser amounts of volatile monoterpenes in *J. osteosperma* (43.64%) vs. *J. monosperma* (78.50%). Thus, the *J. osteosperma* oil is much less volatile than that of *J. monosperma* and coupled with the leaf glands being deeply embedded in the leaves (Fig. 1), leads to less loss of the individual components when exposed to RT conditions than found in *J. monosperma.* It is likely that if woodrats cache *J. osteosperma* leaves in their middens, those leaves will not lose their oils as quickly as leaves of *J. monosperma.*

Examination of the colors of the leaves of *J. osteosperma* shows yellowing of the 4h RT and 18h RT treatments (Fig. 11) that are very similar to that seen in the *J. monosperma* leaves (Fig. 6). As in the case of *J. monosperma* leaves, exposure to RT for up to 18h does not seem to induce any artifacts into the oil, except for the loss of the more volatile monoterpenes.

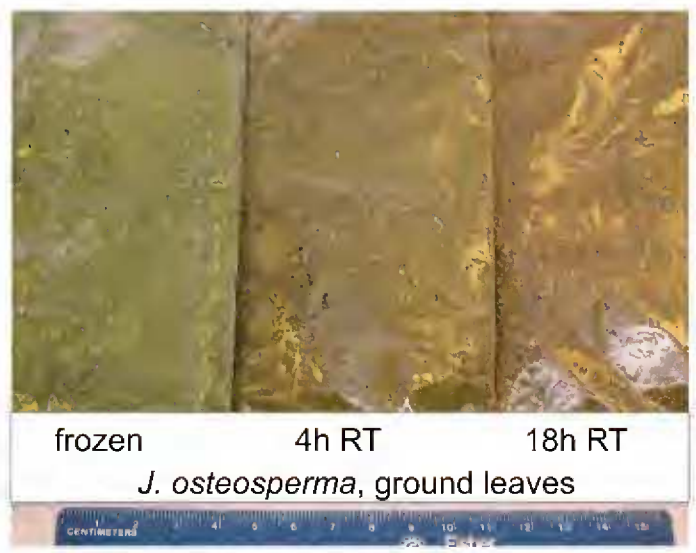

Fig. 11. Colors of ground-frozen, ground-4h RT and ground-18h RT *J. osteosperma* leaves.

CONCLUSION

The effects of sample preparation on yields and composition of volatile terpenoids were examined for oils from *Juniperus monosperma* and *J. osteosperma* leaves, obtained by 24 h steam distillation from intact, ground-frozen, ground- 4h RT, and ground-18h RT leaves. For *J. monosperma*, the total oil yield was largest from the ground-frozen (4.48%), then declined in the 4h RT (4.19%) and

18h RT (2.51%), with yield from intact leaves being intermediate (3.46%). The major component, α-pinene, declined from 22.7 mg/g to 5.8 mg/g upon exposure to RT for 18h. For *J. osteosperma*, the total oil yield was also largest from the ground-frozen (8.9%), then declined in the 4h RT (5.3%) and 18 h RT (4.7%) treatments, with yield from intact leaves being intermediate (5.63%). The major component, bornyl acetate, declined from 14.4 mg/g to 10.0 mg/g upon exposure to RT for 18h. Sabinene declined from 11.3 mg/ g to 3.5 mg/g after exposure to RT for 18h.

The loss of volatile terpenes appears to be mostly effected by differences in total amounts of volatile monoterpenes in *J. osteosperma* (43.64%) and *J. monosperma* (78.50%) and the position of the oil glands (*J. osteosperma*, deeply embedded in the leaves vs. *J. monosperma*, near the leaf surface). *Juniperus monosperma,* with more volatile leaf oil and oil glands near the leaf surface, was much more affected by exposure to RT for 18h than *J. osteosperma* with deeply embedded oil glands, and less volatile oil.

Interestingly, even though the ground leaves were yellowed (brownish) by exposure to RT for up to 18h, this did not seem to induce any artifacts into the oil, except for the loss of the more volatile monoterpenes.

The differences in terpene content and volatility between *J. monosperma* and *J. osteosperma* may help explain differences seen in the woodrat species' tolerance to and preference for specific juniper species.

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Table 1. Comparison of *J. monosperma* leaf oils obtained from leaves that were: intact, ground-frozen, ground-4h RT and ground-18h RT. signif. = significance level, P 0.05 = *, P 0.01 = **; ns = non significant, nt = not tested. Data values on a line that share a common letter are not significantly different. Components in boldface are data on a mg/g DW basis.

KI	component tested	intact leaves	ground, frozen, %	ground, 4h, RT	ground 18 h RT	signif.
	oil yields, 24h dist. - % DW	3.46% b	4.48% a	4.19% ab	2.51% c	**
	oil yields, 24h dist. - mg/g DW	**34.6 b**	**44.8 a**	**41.9 ab**	**25.1 c**	**
921	tricyclene values as % total oil	0.1%	0.2%	0.2%	0.1%	nt
924	α-thujene	t	t	t	t	nt
932	α-pinene	50.8 b	61.8 a	63.3 a	22.9 c	**
	α-pinene, mg/g DW	**17.5 b**	**22.7 a**	**25.5 a**	**5.8 c**	**
945	α-fenchene	0.1	0.1	0.1	t	nt
946	camphene	0.3	0.3	0.3	0.1	nt
969	sabinene	0.1	0.1	0.1	0.1	nt
974	β-pinene	0.9 a	0.9 a	0.9 a	0.4 b	**
988	myrcene	1.4 a	1.6 a	1.5 a	0.9 b	**
1001	δ-2-carene	0.1	0.1	0.1	0.1	nt
1002	α-phellandrene	0.7 a	0.7 a	0.7 a	0.5 b	*
1008	δ-3-carene	2.1 a	2.4 a	2.5 a	1.3 b	**
1014	α-terpinene	0.1	0.1	0.1	0.1	nt
1020	p-cymene	0.3	0.3	0.3	0.2	nt
1024	limonene	2.0 a	2.1 a	1.9 a	1.2 b	**
1025	β-phellandrene	6.0 a	5.9 a	5.6 a	3.7 b	**
1044	(E)-β-ocimene	0.3	0.3	0.3	0.2	nt
1054	γ-terpinene	0.5	0.5	0.5	0.4	nt
1086	terpinolene	1.1 a	1.1 a	1.0 a	0.8 b	*
1100	linalool	t	t	t	0.1	nt
1122	cis-p-menth-2-en-1-ol	0.2	0.1	0.1	0.3	nt
1136	trans-p-menth-2-en-1-ol	0.2	0.1	0.1	0.2	nt
1141	camphor	0.3	0.3	0.2	0.4	nt
1165	borneol	t	t	t	0.2	nt
1174	terpinen-4-ol	0.3	0.3	0.2	0.5	nt
1186	α-terpineol	0.5 b	0.3 c	0.3 c	0.7 a	**
1207	trans-piperitol	0.1	0.1	t	0.2	nt
1249	piperitone	0.1	t	t	0.2	nt
1274	pregeijerene B	2.2 a	1.7 b	1.3 c	1.8 b	**
1284	bornyl acetate	0.4 b	0.5 b	0.4 b	0.8 a	**
1289	thymol	t	t	t	0.2	nt
1396	duvalene acetate	t	t	t	0.1	nt
1417	(E)-caryophyllene	0.2	0.4	0.4	0.7	nt
1452	α-humulene	0.1	0.2	0.2	0.4	nt
1489	β-selinene	t	t	t	0.2	nt
1498	α-selinene	t	t	t	0.1	nt
1500	α-muurolene	t	t	t	0.3	nt
1517	nootkatene	0.4	0.3	0.3	1.0	nt
1533	trans-cadina-1,4-diene	0.2	0.2	0.2	0.7	nt
1548	elemol	4.1 b	2.4 c	2.4 c	8.9 a	**
	elemol mg/g DW	**1.7 b**	**1.1 c**	**1.0 c**	**2.2 a**	**
1566	germacrene B	0.4 b	0.4 b	0.4 b	1.2 a	**
1629	eremoligenol	t	t	t	0.2	nt
1630	γ-eudesmol	3.1 b	2.4 bc	2.2 c	7.3 a	**
1640	epi-α-muurolol	0.2	0.2	0.1	0.6	nt
1649	β-eudesmol	8.1 b	4.3 c	4.4 c	14.6 a	**
	β-eudesmol mg/g DW	**2.8 b**	**1.9 c**	**1.8 c**	**3.7 a**	**
1652	α-eudesmol	4.7 b	3.3 c	3.2 c	11.7 a	**
1668	1-propanone, 1-(2,4-dimethoxy phenyl-)	0.3 b	0.2 c	0.2 c	0.7 a	**
1792	8-α-acetoxyelemol	4.4 b	2.5 c	2.8 c	6.8 a	**

Table 2. Comparison of *J. osteosperma* leaf oils obtained from leaves that were: intact, ground-frozen, ground-4h RT and ground-18h RT. F signif. = F significance, P= 0.05 = *; ns = non significant, nt = not tested. Components in boldface are data on a mg/g DW basis.

KI	component tested	intact leaves	ground, frozen, %	ground, 4h, RT	ground 18 h RT	F signif.
	oil yields, 24h dist. - % DW	5.63% b	8.90% a	5.30% b	4.70% b	**
	oil yields, 24h dist. - mg/g DW	**56.3 mg b**	**89.0 mg a**	**53.0 mg b**	**47.0 mg b**	**
921	tricyclene	0.9 b	1.34 a	1.1 b	0.9 b	**
924	α-thujene	0.6	0.8	0.7	0.5	nt
932	α-pinene	7.3 c	10.5 a	8.9 b	6.6 c	**
	α-pinene, mg/g DW	**4.1 b**	**9.3 a**	**4.7 b**	**3.1 c**	**
946	camphene	1.0 ab	1.3 a	1.2 ab	0.9 b	*
953	thuja-1,4-diene	0.1	0.1	0.1	0.1	nt
969	sabinene	8.6 c	12.7 a	10.7 b	7.4 c	**
	sabinene, mg/g DW	**4.8 b**	**11.3 a**	**5.7 b**	**3.5 c**	**
974	β-pinene	0.2	0.2	0.2	0.2	nt
988	myrcene	2.5	2.9	3.0	2.6	ns
1002	α-phellandrene	0.2	0.3	0.3	0.3	nt
1008	δ-3-carene	0.1	0.2	0.2	0.2	nt
1014	α-terpinene	1.8 a	1.2 b	1.6 a	1.5 a	**
1020	p-cymene	1.5	1.3	1.5	1.5	ns
1024	limonene	3.9	4.1	4.4	4.1	ns
1025	β-phellandrene	2.5	2.8	3.0	2.7	ns
1044	(E)-β-ocimene	0.2	0.3	0.3	0.3	nt
1054	γ-terpinene	3.3 a	2.1 b	2.8 a	2.8 a	**
1065	cis-sabinene hydrate	0.7	0.4	0.2	0.3	nt
1086	terpinolene	1.4	1.1	1.3	1.3	ns
1098	trans-sabinene hydrate	0.9	0.5	0.4	0.5	nt
1102	isopentyl-isovalerate	0.3	0.2	0.2	0.2	nt
1112	methyl butanoate, 3-methyl-3-butenyl-, 3-	0.4	0.3	0.3	0.3	nt
1122	cis-p-menth-2-en-1-ol	0.6	0.4	0.4	0.4	nt
1141	camphor	9.0	8.5	9.6	10.5	ns
	camphor	**5.1 b**	**7.6 a**	**5.1 b**	**4.9 b**	**
1145	camphene hydrate	0.6	0.5	0.6	0.7	nt
1154	sabina ketone	0.3	0.3	0.3	0.4	nt
1165	borneol	1.6 b	1.7 b	1.8 b	2.5 a	**
1174	terpinen-4-ol	8.6 a	5.1 c	6.6 b	7.4 b	**
1179	p-cymen-8-ol	0.4	0.3	0.3	0.3	nt
1186	α-terpineol	0.5	0.3	0.4	0.4	nt
1204	verbenone	0.6	0.4	0.5	0.5	nt
1215	trans-carveol	0.5	0.4	0.4	0.6	nt
1239	carvone	0.5	0.2	0.2	0.3	nt
1284	bornyl acetate	17.5 b	16.1 b	18.8 ab	21.3 a	*
	bornyl acetate, mg/g DW	**9.9 b**	**14.3 a**	**10.1 b**	**10.0 b**	**
1325	p-mentha-1,4-dien-7-ol	0.5	0.3	0.4	0.5	nt
1417	(E)-caryophyllene	t	0.3	0.3	0.3	nt
1451	trans-muurola-3,5-diene	t	0.1	0.1	0.1	nt
1452	α-humulene	t	0.1	0.1	0.1	nt
1480	germacrene D	t	0.1	0.1	0.1	nt
1500	α-muurolene	t	0.1	0.1	0.1	nt
1513	γ-cadinene	t	0.1	0.1	0.1	nt
1522	δ-cadinene	0.6	0.9	0.8	0.9	nt
1548	elemol	8.2 a	5.2 b	4.2 b	4.8 b	**
	elemol, mg/g DW	**4.6 a**	**4.6 a**	**2.2 b**	**2.3 b**	**
1559	germacrene B	t	0.1	0.1	0.1	nt
1574	germacrene D-4-ol	t	0.3	t	0.1	nt
1630	γ-eudesmol	0.6 b	0.9 a	1.0 a	1.0 a	**
1647	cubenol	0.6	0.6	0.5	0.6	nt

KI	component tested	intact leaves	ground, frozen, %	ground, 4h, RT	ground 18 h RT	F signif.
1649	β-eudesmol	0.6	0.7	0.6	0.7	nt
1652	α-eudesmol	0.7	0.7	0.6	0.7	nt
1652	α-cadinol	0.6	0.7	0.6	0.7	nt
2087	abietadiene	t	0.4	0.3	0.3	nt
2315	abieta-7,13-dien-3-one	4.7 a	4.9 a	3.8 b	4.8 a	*